14

AN EXAMINATION FOR A BIOLOGICAL CLOCK IN *CERATOCYSTIS ULMI*

A Thesis

Presented to

The Graduate Division

Drake University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

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by

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January 1965

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CHAPTER I

INTRODUCTION

Science has been concerned with the rhythmic periodicity of organisms for over 200 years. The organisms with which man has worked have ranged from protozoans[1] to man[2] in the animal kingdom and from algae[3] to flowering plants[4] in the plant kingdom. Even with this diversity of organisms, little work has been done showing periodicity in fungi as indicated by Erwin Bunning, "circadian rhythms ... are still to be found in many fungi."[5] Despite this small amount of work, it is probable that many fungi do possess a biological clock. Frank A. Brown, Jr. has stated, "It has become

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[1]J. W. Hastings, "Unicellular Clocks," Annual Review of Microbiology, XIII (November, 1959), 297-312.

[2]M. C. Lobban, "The Entrainment of Circadian Rhythms in Man," Biological Clocks, Leonora Frisch, editor (Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960), pp. 325-332.

[3]Dieter Maller, "Development of Sporangia and Lunar Periodicity on Brown Algae," Botanical Marina, IV (February, 1962), 140-162.

[4]F. A. Brown, Jr., "Living Clocks," Science, CXXX (December 4, 1959), 1535-1544.

[5]E. Bunning, "Opening Address: Biological Clocks," Biological Clocks, Leonora Frisch, editor (Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960), pp. 1-9.

increasingly evident in recent years that all organisms are rhythmic systems."[1]

Concentric, alternate sparse and dense growth bands occurred in Ceratocystis ulmi, when grown in alternate light-dark cycles. This suggested the possibility that Ceratocystis ulmi could be studied for a biological clock because of its photoperiodicity. A study to determine whether a biological clock occurred in Ceratocystis ulmi appeared appropriate and in addition would bring more light concerning periodicity in one of the less studied groups, the fungi.

The purpose of this study was to demonstrate the presence or absence of a biological clock in Ceratocystis ulmi by testing for periodic oxygen consumption after being synchronized with a twelve hour light-dark sequence.

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[1]F. A. Brown, Jr., "Extrinsic Rhythmicality: A Reference Frame for Biological Rhythms Under So Called Constant Conditions," Annals of the New Year Academy of Sciences, XCVIII (October, 1962), 775.

CHAPTER II

REVIEW OF THE LITERATURE

Ceratocystis ulmi, the perfect stage of *Graphium ulmi* (Buisman, 1922),[1] is a known cause of Dutch Elm Disease.[2] This organism's mycelium grows concentrically with dense and sparse rings when placed in a daily light-dark cycle. The phenomenon has been observed commonly in the laboratory.

This diurnal pattern has been found in other fungi, such as growth rate in *Neurospora*[3] and sporulation of *Pilobolus*.[4] In *Pilobolus*, sporulation occurred when placed in an alternating 12 hours of light and 12 hours of darkness (LD-12:12). This length of periodicity is referred to as a circadian rhythm, a free running biological rhythm which is

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[1] John Hunt, "Taxonomy of Genus *Ceratocystis*," *Lloydia*, XIX (January, 1958), 1-58.

[2] C. Moreau, "*Ceratocystis ulmi*," *Revuede Mycalogie Supplementary Colonial*, XVII (1952), 22.

[3] Colin S. Pittendrigh, V. G. Bruce, N. S. Rosenswevy and M. L. Rubin, "Growth Patterns in Neurospora," *Nature*, CLXXXIV (July 18, 1959), 169-171.

[4] E. R. Uebelmesser, "Ueberden endonomer Tagesrhythmus der Sporangientraegerbildung von Pilobolus," *Archiv fur Mikrobiologie* XX (February, 1954), 1-33.

approximately the 24 hour period of the earth's rotation.[1] Circadian rhythms can be shifted when the LD cycle is reversed from the normal, making it light when it is ordinarily dark and dark when ordinarily light. Under this reversal of conditions, it has been observed that some organisms will completely rephase to the new LD cycle.[2] Normally organisms have only a circadian type of rhythm even if the light that is being used to set the rhythm occurs at a cycle other than a 24 hour cycle. Using LD periods of 6:6; 7:7; 8:8; and 16:16 has been demonstrated to set 12, 14, 16, and 32 hour periodicities respectively in the bioluminescence of a dinoflagellate[3] and LD 8:8 and 9:9 set 16 and 32 hour cycles of leaf movements in bean plants.[4] As soon as constant

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[1]Collin S. Pittendrigh, "Circadian Rhythms and the Circadian Organization of Living Systems," Biological Clocks, Leonora Frisch, editor (Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960), pp. 29-45.

[2]Victor G. Bruce, "Environmental Entrainment of Circadian Rhythms," Biological Clocks, Leonora Frisch, editor (Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960), pp. 29-45.

[3]J. W. Hastings and B. M. Sweeny, "A Persistent Diurnal Rhythm of Luminescence in Gonyaulax polyedra," Biological Bulletin, CXV (December, 1958),

[4]E. Bunning, "Endogenous Diurnal Cycles of Activity in Plants," Rhythmic and Synthetic Processes in Growth, G. Rudnik, editor (Princeton: Princeton University Press, 1957), pp. 111-126.

conditions are resumed such as continuous darkness or dim light, the organisms return to their normal 24 hour rhythm. There is one exception according to Wilkins,[1] one species of algae has been shown to have a persistent rhythm of 12 and 17.5 hours for three days in continuous darkness when set with LD 6:6 and 10:5:7 respectively.

The using of a stimulus such as light to set a clock or biological rhythm in an organism is referred to as a synchronizing agent. The terms Zeitgeber, time cue, and entrainer are also used.[2] To be a true biological clock, possessing an endogenous rhythm, the rhythm must be free running without the stimulus of the synchronizer or any other external periodic stimulus.[3] If an external stimulus is required to keep the clock going, it is referred to as an exogenous rhythm.[4]

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[1]Malcolm B. Wilkins, "The Effect upon Plant Rhythms," Biological Clocks, Leonora Frisch, editor (Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960), pp. 115-129.

[2]Jurgen Aschoff, "Exogenous and Endonous Components in Circadian Rhythms," Biological Clocks, Leonora Frisch, editor (Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960), pp. 11-26.

[3]Ibid.

[4]Ibid.

The two most commonly used synchronizers are light and temperature, but others including chemicals have been used.[1] Light is a very strong synchronizer and can be used to set a clock where other synchronizers can not. In fact, periodic flashes of light lasting 1/2000 second have been used to reset the time of periodicity in Euglena and Drosophilia.[2] Also it has been shown that continuous bright light will damp out a periodicity in such organisms as Pilobulus[3] where as with many organisms periodicity persists only in darkness. The color of light may have an effect depending on the type of organism. Using red light the periods are about three to five hours longer than in constant darkness for green plants,[4] but dim red light has no effect

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[1]C. L. Ralph, "Modifications of Activity Rhythms of Periplaneta americana (L) Induced by Carbon Dioxide and Nitrogen," Physiological Zoology, XXXII (January, 1959), 57-62.

[2]C. L. Pittendrigh, V. G. Bruce, "Daily Rhythms as Coupled Oscillator Systems and their Relation to Thermoperiodism and Phototropism," Photoperiodism and Related Phenomena in Plants and Animals, C. Withrow, editor (Washington: American Association for the Advancement of Science, 1959), 475-505.

[3]Uebelmesser, op. cit., 1-33.

[4]E. Bunning, "Opening Address: Biological Clocks," Biological Clocks, Leonora Frisch, editor (Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960), pp. 1-9.

in setting or damping out a periodicity in Neurospora.[1]

The effect of temperature as a synchronizer has been shown to have various results depending on the type of organism being tested.[2] Generally circadian rhythms are synchronized by a 24 hour temperature cycle when the synchronizing temperature has a range of at least 5 degrees centigrade.[3] Also it has been found that the high temperature phase corresponds to the light phase of an organism's periodicity whereas the low temperature phase corresponds with the dark phase.[4] Sporulation in Pilobolus was synchronized on a 12 hour 21°C, and 12 hour 15°C while in continuous darkness. The alternating temperature set the clock.[5]

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[1]Colin S. Pittendrigh, V. G. Bruce, N. S. Rosenswevy and M. L. Rubin, "Growth Patterns in Neurospora," Nature, CLXXXIV (July 18, 1959), 169-171.

[2]Victor C. Bruce, "Environmental Entrainment of Circadian Rhythms," Biological Clocks, Leonora Frisch, editor (Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960), pp. 29-40.

[3]Ibid.

[4]Ibid.

[5]A. Schmidle, "Die Tagesperiodizitat der Asexuellen Reproduktion von Pilobolus sphaerosporus, Archiv fur Mikrobiologie, XVI (May, 1959), 80-100.

Another fungus, Neurospora has been shown to have a temperature independent periodicity.[1]

F. A. Brown, Jr. has demonstrated rhythms running with period lengths that coincide with externally occurring events of the same period length. He has shown that potatoes metabolize with a period length of 24.8 hours, a lunar day,[2] and mice have average peaks of activity periods in addition to lunar days, with synodic monthly cycles (29.5 days).[3] Also a tidal rhythm of 12.4 hours has been found in the running of crabs in constant conditions.[4] When these crabs were moved to a different part of the country they adjusted their rhythm to the time when there should be high tide in that part of the country. Brown has demonstrated that the

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[1]Pittendrigh, loc. cit.

[2]Frank A. Brown, Jr., "An Exogenous Reference Clock for Persistent Temperature-Independent, Liable Biological Rhythms." Biological Bulletin, CXV (August, 1958), 81-100.

[3]Frank A. Brown, Jr., J. Shriner and C. L. Ralph, "Solar and Lunar Rhythmocity in the Rat in Constant Conditions and the Mechanism of Physiological Time Measurement," American Journal of Physiology, CLXXXIV (March, 1956), 491-496.

[4]Franklin H. Barnwell, "Observations and Daily Tidal Rhythms in Some Fiddler Crabs from Equatorial Brazil," Biological Bulletin, CXXV (December, 1963), 399-415.

earth's magnetism,[1] eletrostatic fields around the earth[2] and gravitational pull of the moon[3] can be detected by certain organisms and so could be possible synchronizers. If Brown's proposals are correct for all organisms that have been used to show an endogenous biological clock, it may be possible that all these periodicities are exogenous since a true clock requires it persists without an external stimulus. Investigators Bunning[4] and Pittendrigh[5] differ with Brown's conclusions and believe that these are not synchronizers; that the clocks demonstrated are truly endogenous. Most present investigations continue to ignore other possible synchronizers suggested by Brown and refer to constant conditions as being light and temperature, and in some cases atmospheric pressure.

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[1]F. A. Brown, Jr., "Responses of the Planarian, Dugesia and the Protozoan Paramecium, to Very Weak Horizontal Magnetic Fields," Biological Bulletin, CXXIII (October, 1962), 264-281.

[2]F. A. Brown, Jr., "Response of the Planarian, Dugesia to Very Weak Horizontal Electrostatic Fields," Biological Bulletin, CXXIII (October, 1962), 282-294.

[3]Brown, loc. cit.

[4]E. Bunning, "Opening Address: Biological Clocks," Biological Clocks, Leonora Frisch, editor (Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960), pp. 1-9.

[5]Colin S. Pittendirgh, "Circadian Rhythms and Circadian Organization of Living Systems," Biological Clocks, Leonora Frisch, editor (Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960), pp. 159-184.

Many different indicators have been used to demonstrate the presence of a biological clock. A few other examples are: color changes in fiddler crabs;[1] and phototaxis of Euglena.[2] All of the examples represent some physiological activity but do not necessarily represent the clock itself. As is pointed out by various investigators, the hands of the clock should not be confused for the clock itself.[3, 4, 5]

One way of proving the presence of a biological clock requires that the organism in "constant conditions" has a persisting periodicity, "but with a frequency deviating by a certain, more or less constant, amount from that of the

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[1]Franklin H. Barnwell, "Observations and Daily Tidal Rhythms in Some Fiddler Crabs from Equatorial Brazil," Biological Bulletin, CXXV (December, 1963), 399-415.

[2]V. G. Bruce, C. S. Pittendrigh, "Resetting the Euglena Clock with a Single Light Stimulus," American Naturalist, XCII (September-October, 1958), 295-306.

[3]E. Bunning, op. cit., 3.

[4]J. W. Hastings, "Unicellular Clocks," Annual Review of Microbiology, C. E. Clifton, editor XIII (Palo Alto, California, Annual Reviews, Inc., 1959), 297-312.

[5]F. A. Brown, Jr., "Response to Pervasive Geophysical Factors and the Biological Clock Problem," Biological Clocks, Leonora Frisch, editor (Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960), pp. 57-71.

earth-rotation."[1] Therefore in order to determine the presence or absence of a biological clock in Ceratocystis ulmi, it was grown in constant conditions except when the synchronizer was used at which time the organism was grown in a LD (12:12). The "clock" or the "hands" used for determining periodicity was periodic metabolism as indicated by oxygen consumption.

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[1]Jurgen Aschoff, "Enogenous and Endogenous Components on Circadian Rhythms," Biological Clocks, Leonora Frisch, editor (Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960), pp. 12.

CHAPTER III

METHODS AND MATERIALS

The oxygen consumption was measures with a continuous recording apparatus (Figure 1) modified after the model designed by F. A. Brown, Jr.[1] The respirometer is based upon measurement of decreasing buoyancy of a diver as a function of oxygen utilization. Aerobic respiring took up oxygen and released carbon-dioxide. The diver's oxygen reservoir (see Figure 2) supplied the oxygen and the carbon-dioxide was absorbed by potassium-hydroxide. The recording apparatus recorded the gradual sinking of the diver on a slow turning drum.

The diver is composed of three basic parts (Figure 2), the petri plate, the holder and the oxygen reservoir. The bottom half of a 100 mm plastic petri plate with a 1/8 inch of potato dextrose agar (Bacto) in the bottom was used. The agar was autoclaved for 20 minutes at 20 pounds pressure and poured into sterile petri plates where it was stored in a refrigerator until used. The bottom half of the petri plate was secured to the holder with florist clay obtainable from Beagle Manufacturing Company, Inc., Pasadena, California.

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[1] F. A. Brown, Jr., "A Simple Automatic, Continuous-Recording Respirometer," Review of Scientific Instruments, XXV (1954b), 415-417.

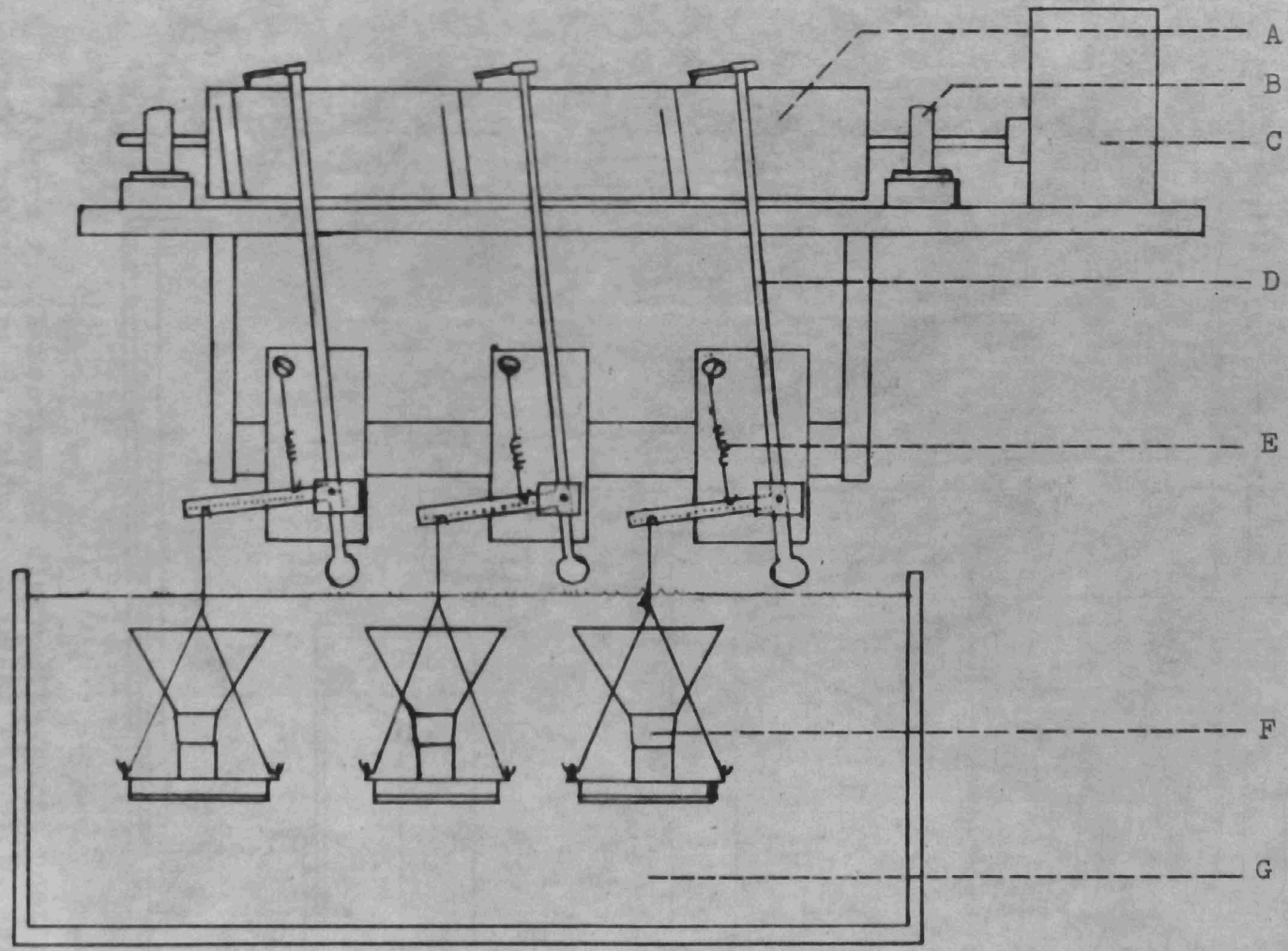

Figure 1. Recording apparatus with divers.
A. recording drum; B. bearing; C. clock motor;
D. recording arm; E. spring; F. diver; G. water

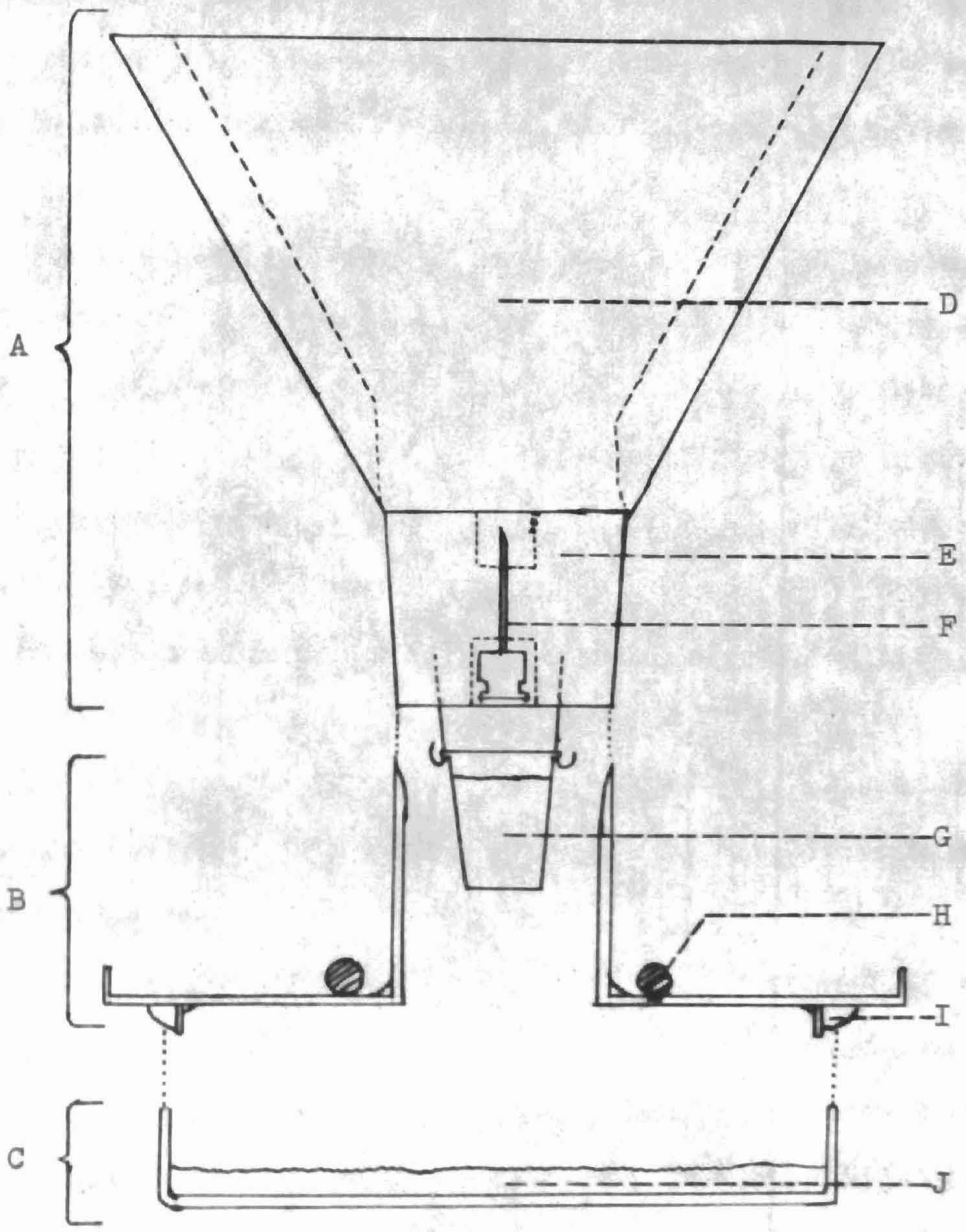

Figure 2. X-Section of diver with parts unattached. A. oxygen reservoir; B. holder; C. petri plate; D. saran bag; E. rubber stopper; F. hypodermic needle; G. absorbent vessel; H. lead ballast; I. clay; J. agar

For purposes of making a secure seal between the petri plate and holder, the clay was heated on the holder to make it soft and gummy just before the petri plate was attached. Brackets were made to hold the petri plate to the holder, but were later discarded because it was found the clay held the plate securely.

The holder (Figure 2) was made out of galvanized sheet metal bent 1/8 inch on each edge for rigidity. A one inch diameter copper tubing one and a half inches long was soldered to the top of the holder. This tubing acted as a holder for the oxygen reservoir. A 1/8 inch galvanized strip was soldered in a circle to the bottom of the holder so that it was 1/8 inch smaller in diameter than the petri plate to be used. This strip helps add surface area for the florist clay to stick so that a tighter seal will be made between petri plate and holder. The entire holder was painted with black Rustoleum paint.

The oxygen reservoir (Figure 2) is composed of a rubber stopper and a saran bag. The #6½ rubber stopper was drilled out 1/3 of the way at both ends in order to recess the #27 hypodermic needle, diminishing the possibility of puncturing the collapsing saran bag. The saran bag is made with a double layer of saran, Saran Wrap, manufactured by Dow Chemical Company, Midland, Michigan, which is folded

over at the edges twice and sealed with tile paste, Tile Paste manufactured by Schalk Chemical Company, Chicago, Illinois, and clear water resistant scotch tape, Scotch Brand Tape manufactured by 3M Minnesota Mining and Manufacturing Company, St. Paul, Minnesota. The tile paste was used to secure a permanent air tight seal between the saran bag and rubber stopper. Attached to the bottom of the rubber stopper was a small plastic vial suspended with nichrome wire. This vial was used to hold two cc of saturated potassium hydroxide, a carbon-dioxide absorbent. There was an adequate seal between the rubber stopper and holder when the stopper was placed in tightly so no additional sealer was used. Fifty cubic centimeters of commercially prepared pure oxygen was put into the saran bag through the needle with a hypodermic syringe. A heavy cotton thread from the recording arm, suspended the diver in the water by attaching to the holder. The divers were too light to sink. Lead ballasts were added to the divers until they weighed two grams. This gave each diver the same zero point at the beginning of the experiment.

The recording apparatus (Figures 1 and 3) is adopted from Brown.[1] A short description is given to note the differences from Brown's apparatus and to give an understanding

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[1]Brown, loc. cit.

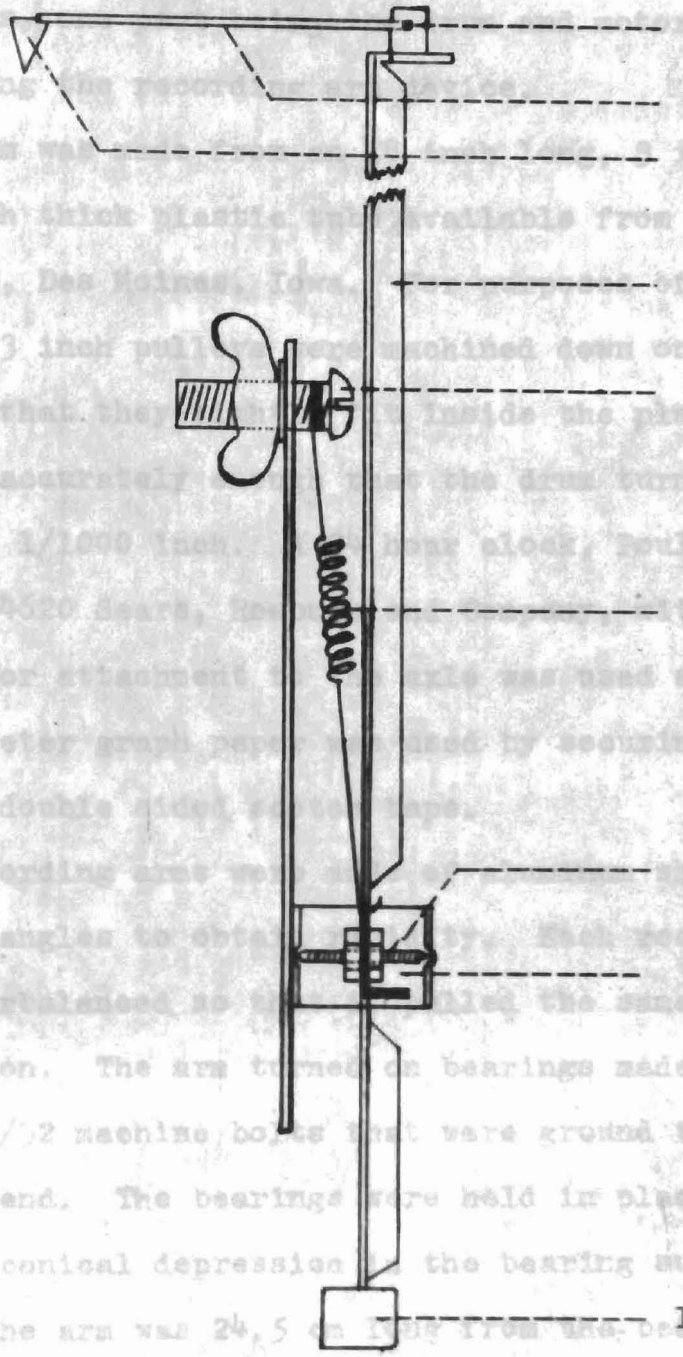

Figure 3. Side view of recording arm holder and recording arm. A. bearing; B. flexible arm; C. recording pen; D. recording arm; E. tension adjustment screw; F. stainless steel spring; G. bearing; H. bearing support; I. counterweight

as to how the machine works. The recording was composed of two basic parts, one part being the drum and motor and the other part being the recording arm device.

The drum was made from an 18 inch long, 3 inch diameter $\frac{1}{4}$ inch thick plastic tube available from Van Horn Plastics, Inc., Des Moines, Iowa. For purposes of holding the drum, two 3 inch pulleys were machined down on a $\frac{1}{2}$ inch steel axle so that they tightly fit inside the plastic tube. This was done accurately enough that the drum turned within a tolerance of 1/1000 inch. A 24 hour clock, Poultry House Time Switch, #4620 Sears, Roebuck and Company, with a modification for attachment to the axle was used as the motor. Millimeter graph paper was used by securing it to the drum with double sided scotch tape.

The recording arms were made of aluminum sheeting bent at right angles to obtain rigidity. Each recording arm was counterbalanced so that it pulled the same regardless of position. The arm turned on bearings made from 1 inch long, 3/32 machine bolts that were ground to a point on each end. The bearings were held in place with each end in a conical depression in the bearing supports (Figure 3). The arm was 24.5 cm long from the bearing to the end with the extension for the pen. The right angle of the arm was 10 cm long, but the exact location of the spring

and diver attachment varied depending on the leverage desired. The pen was fixed to a 7.5 cm extension from the recording arm, made movable by the use of a bearing (Figure 3). The writing pens were homemade but styled after the type typically used on barographs. Weak stainless steel springs were arranged as in Figure 1 and 3 to be adjustable for purposes of putting tension on the recording arm.

Two 20-watt, cool-white fluorescent lamps were used for lighting. Approximately forty foot candles were measured at the divers. The turning on and off of the lights was done with a clock switch.

The temperature was kept at 23°C ($\pm\frac{1}{2}$°) with the use of 100 watt aquarium heaters.

Barometric pressure corrections were made on the data based on recordings of a barograph.

The organism used in this investigation, Ceratocystis ulmi, was obtained from Roy H. McFall who in turn received it from the Dutch Elm Diagnostic Laboratory at Des Moines, Iowa where it was isolated and identified from a sample of diseased elm wood. Inoculations were always taken from the outer dark ring of four day old stock cultures and were always made in a transfer chamber which was wiped with concentrated Lysol before using. The stock culture was kept at 22°C ($\pm$1°) with LD (12:12).

LD (12:12) was used during the first 3 days after the divers were innoculated and placed in the water. The light cycle began at 8:35 pm and ended at 8:50 am for three series of experiments. The light cycle was reversed for the fourth experimental series which began at 8:45 am and ended at 8:55 pm. In all experimental series, the last five days were run in complete darkness (DD). The experiment was run in a basement room that had no windows and only one door which was kept closed except when entering or leaving. The experiment was checked at every light period between 9 and 10 pm and once at the end of 72 hours of DD for the purpose of filling pens and resetting the recording arms if necessary. When entering the room during DD, a flashlight with two sheets of white paper covering the lens was used for light. Great care was taken not to shine the light on the divers with the flashlight or to let light into the room when entering or leaving.

Records made by the recording apparatus were transposed to numbers and graphs for purposes of comparing LD and DD periodic oxygen consumption.

CHAPTER IV

RESULTS AND INTERPRETATION OF DATA

A preliminary investigation of the growth of Ceratocystis ulmi revealed that the colonies were typically circular when grown on potato dextrose medium in conditions associated with the diver. But the typical concentric ring pattern which was common when grown on petri plates in a LD 12:12 was not apparent or only one ring could be detected, when grown in the diver for three days in a LD 12:12 and five days DD. This pattern of growth was typically what happened when Ceratocystis ulmi was started on petri plates by the stab technique, the procedure used in this experiment. Normally no rings were seen until the end of the third day. After the third day the pattern became sequentially more pronounced. The growth appeared to be bunched rather than ringed the first few days. From this observation, it appeared that the continuence of the concentric rings in constant conditions did not exist. Under these experimental conditions, the concentric ring growth was not controlled by a biological clock. More investigation would be necessary before any definite conclusions covering this aspect of study could be drawn.

To test the apparatus, one group of divers was placed in the machine without inoculating the agar. After three

days, a minute amount of sinking by the divers was detected but with a constant rate.

Barometric changes during the three days had no apparent effect on the diver position. With further calculations using the largest barometric pressure changes recorded during the entire experimentation, the largest one hour change of barometric pressure changed the diver weight in water 0.003 grams and the largest total 24 hour change changed the diver weight 0.014 grams. This amount of change, when considered per hour, is not enough to cause a change in recording because of the spring tension used. No corrections for barometric pressure were necessary.

Four "runs" or experimental series of eight days each were made using four divers the first run and six divers the remaining three runs. Due to mechanical failures of the apparatus or contamination of the agar medium, not all the cultures in different divers could be used for data. Three sets of data were used from run one, five from run two, four from run three and four from run four.

The first three runs were in LD 12:12 with the illumination beginning at 8:35 pm[1] and extinguished at 8:40 am. The illumination was reversed on the fourth run. Illumination was started at 8:45 am and extinguished at 8:55 pm.

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[1]All time is given in DST.

Readings were made from the recording paper by measuring from a base line. The relative amount of oxygen for each hour was determined. No attempt was made to convert the readings made from pen movements into actual amounts of oxygen consumed, but the readings were used as a relative indicator of oxygen consumption. As an aid in making the readings, a three inch circular hand lens on a base was used. The large end of a paper cone was attached to the rim of the lens. The small end of the cone had an opening through which the records were viewed. The cone reduced parallax when making a reading. Using the hand lens and a bright light, readings were possible with an accuracy of plus or minus 0.1mm.

All data was recorded as relative amounts of oxygen consumed during a given hour. An arithmetric mean for each hour of the eight day run was obtained for all u of each run. The runs were computed separately to show any similarities or differences that might be encountered by running the experiment at different times of the month. Tables I, II, III, and IV give these average differences of oxygen consumption for each hour of the day. In Tables I-IV, each day of the run is recorded horizontally and the amount of pen movement for each hour of the day is recorded vertically. For example, Run I (Table I) at 9:00 am on day

TABLE I

AN ARITHMETIC MEAN OF DATA FROM THREE DIVERS OF RUN I. EACH NUMBER INDICATES RELATIVE OXYGEN CONSUMPTION FOR EACH HOUR OF THE EIGHT DAY RUN

Time	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Mdnt.	.040	.035	.037	.037	.067	.033	.057	.147
1:00	.030	.023	.050	.047	.027	.037	.070	.057
2:00	.010	.063	.060	.037	.027	.060	.053	.050
3:00	.050	.037	.080	.060	.020	.020	.100	.090
4:00	.010	.027	.020	.030	.035	.007	.027	.130
5:00	.015	.040	.043	.030	.030	.037	.060	.107
6:00	.025	.057	.087	.033	.073	.020	.030	.020
7:00	.060	.053	.130	.060	.047	.027	.027	.007
8:00	.045	.123	.087	.033	.080	.050	.167	.033
9:00	.060	.067	.050	.067	.053	.083	.080	.090
10:00	.085	.045	.083	.067	.047	.083	.080	.067
11:00	.035	.070	.047	.047	.027	.100	.050	.110
Noon	.025	.033	.013	.030	.047	.073	.030	.100
1:00	.015	.017	.023	.010	.050	.053	.030	.083
2:00	.010	.013	.017	.017	.017	.027	.023	.063
3:00	.015	.030	.007	.017	.010	.010	.023	.060
4:00	.020	.010	.020	.003	.017	.025	.043	.057
5:00	.020	.000	.013	.023	.007	.020	.047	.070
6:00	.027	.017	.010	.023	.010	.007	.033	.077
7:00	.010	.010	.013	.017	.027	.017	.023	.070
8:00	.010	.023	.010	.003	.043	.030	.037	.067
9:00	.023	.010	.000	.007	.023	.023	.047	
10:00	.000	.080	.023	.010	.010	.030	.013	
11:00	.020	.010	.037	.067	.033	.137	.113	

TABLE II

AN ARITHMETIC MEAN OF DATA FROM FIVE DIVERS OF RUN 2. EACH NUMBER INDICATES RELATIVE OXYGEN CONSUMPTION FOR EACH HOUR OF THE EIGHT DAY RUN

Time	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Mdnt.	.022	.045	.060	.170	.170	.215	.180	.185
1:00	.040	.030	.055	.160	.155	.200	.235	.215
2:00	.025	.025	.020	.050	.080	.090	.190	.195
3:00	.050	.030	.060	.100	.125	.230	.135	.195
4:00	.015	.060	.085	.195	.130	.165	.225	.185
5:00	.045	.065	.030	.095	.160	.165	.225	.270
6:00	.055	.105	.110	.165	.165	.255	.280	.210
7:00	.055	.235	.240	.190	.190	.210	.255	.225
8:00	.075	.290	.325	.235	.195	.205	.285	.235
9:00	.095	.160	.225	.205	.240	.255	.290	.190
10:00	.070	.025	.100	.150	.220	.210	.230	.170
11:00	.035	.080	.125	.145	.175	.170	.190	.195
Noon	.075	.100	.110	.195	.150	.195	.180	.150
1:00	.070	.225	.155	.140	.155	.190	.220	.170
2:00	.050	.160	.120	.110	.125	.215	.215	.110
3:00	.070	.140	.125	.170	.135	.140	.200	.080
4:00	.065	.100	.100	.190	.140	.175	.205	.140
5:00	.055	.100	.140	.125	.180	.220	.240	.110
6:00	.085	.280	.125	.115	.140	.235	.200	.120
7:00	.070	.110	.135	.180	.145	.160	.180	.140
8:00	.050	.080	.125	.180	.205	.285	.220	.130
9:00	.060	.060	.170	.150	.175	.225	.225	.080
10:00	.060	.065	.160	.180	.135	.195	.245	
11:00	.060	.075	.145	.155	.230	.285	.220	

TABLE III

AN ARITHMETIC MEAN OF DATA FROM FOUR DIVERS OF RUN 3. EACH NUMBER INDICATES RELATIVE OXYGEN CONSUMPTION FOR EACH HOUR OF THE EIGHT DAY RUN

Time	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Mdnt.	.010	.010	.083	.138	.250	.363	.400	.530
1:00	.010	.013	.025	.158	.220	.335	.403	.533
2:00	.015	.007	.023	.070	.220	.375	.427	.440
3:00	.013	.015	.045	.075	.140	.373	.375	.443
4:00	.018	.013	.018	.063	.220	.343	.353	.500
5:00	.018	.015	.025	.130	.225	.288	.373	.517
6:00	.028	.045	.053	.105	.313	.385	.388	.503
7:00	.043	.033	.075	.145	.285	.353	.365	.503
8:00	.053	.030	.078	.133	.285	.423	.413	.440
9:00	.038	.048	.078	.175	.255	.383	.473	.517
10:00	.045	.063	.043	.128	.250	.348	.403	.407
11:00	.025	.043	.015	.150	.270	.318	.380	.437
Noon	.038	.035	.045	.120	.263	.345	.390	.383
1:00	.010	.013	.018	.108	.263	.355	.370	.383
2:00	.000	.020	.018	.073	.200	.315	.443	.347
3:00	.000	.007	.018	.113	.203	.360	.483	.373
4:00	.007	.010	.010	.135	.283	.350	.283	.433
5:00	.013	.018	.055	.170	.293	.355	.380	.377
6:00	.010	.010	.030	.138	.243	.413	.433	.377
7:00	.007	.023	.065	.123	.288	.368	.347	.527
8:00	.007	.023	.048	.148	.265	.423	.540	.480
9:00	.000	.028	.088	.160	.303	.442	.373	.390
10:00	.015	.025	.068	.195	.328	.418	.527	.300
11:00	.023	.058	.093	.220	.338	.395	.620	.450

TABLE IV

AN ARITHMETIC MEAN OF DATA FROM FOUR DIVERS OF RUN 4. EACH NUMBER INDICATES RELATIVE OXYGEN CONSUMPTION FOR EACH HOUR OF THE EIGHT DAY RUN

Time	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Mdnt.	.033	.190	.250	.235	.343	.297	.270	.163
1:00	.030	.160	.234	.263	.180	.243	.190	.165
2:00	.038	.150	.270	.278	.247	.287	.253	.165
3:00	.068	.120	.270	.238	.213	.260	.268	.180
4:00	.075	.145	.245	.270	.190	.257	.278	.183
5:00	.100	.153	.235	.225	.220	.237	.237	.135
6:00	.120	.185	.260	.268	.280	.283	.320	.190
7:00	.133	.148	.273	.270	.283	.313	.253	.193
8:00	.138	.205	.268	.285	.263	.350	.267	.200
9:00	.165	.230	.268	.270	.260	.323	.297	.250
10:00	.120	.203	.283	.228	.253	.297	.263	.218
11:00	.098	.143	.245	.260	.207	.273	.223	.178
Noon	.100	.165	.240	.248	.190	.333	.280	.173
1:00	.083	.135	.175	.225	.223	.227	.203	.113
2:00	.073	.160	.170	.188	.243	.317	.197	.108
3:00	.123	.145	.180	.215	.217	.270	.190	.100
4:00	.103	.105	.170	.228	.187	.270	.187	.098
5:00	.085	.120	.205	.185	.197	.247	.143	.075
6:00	.080	.150	.220	.168	.163	.210	.197	.135
7:00	.073	.145	.175	.218	.223	.240	.127	.150
8:00	.085	.175	.230	.223	.307	.355	.180	
9:00	.073	.163	.160	.203	.187	.170	.170	
10:00	.165	.260	.262	.265	.287	.310	.093	
11:00	.168	.290	.280	.273	.310	.330	.150	

five has a relative movement of .053 for that hour. This is a large movement when compared to 8:00 am and 10:00 am of the same day.

Since Ceratocystis ulmi grew during the eight days of experimentation, more oxygen was consumed on the last day than on the first. Correlation of data for each day was accomplished by adjusting the growth factor. Computation was done by making a running average from this data in Tables I, II, III and IV. The percent of oxygen consumed per hour above or below the running average was computed by dividing the running average into the hourly oxygen consumption.

If an oxygen consumption for one hour was computed to be 100%, this would indicate that this hour was the same as the average for the 24 hour period around it. A higher percent than 100 indicated more oxygen was consumed than the average for 24 hours. If this percent was less the reverse would be true. An examination of Table V shows that at 1:00 am of day 6, the oxygen consumption was 101%, nearly average for the 24 hour period of time. But at 11:00 am on the same day, the percent of oxygen consumption was 251% or 2½ times average.

TABLE V

AN HOURLY % OF OXYGEN COMPUTED FROM RUN I*

Time	Day 1 %	Day 2 %	Day 3 %	Day 4 %	Day 5 %	Day 6 %	Day 7 %	Day 8 %
Mdnt.		99	64	192	212	92	111	251
1:00		65	120	151	82	101	140	93
2:00		177	143	120	80	162	107	79
3:00		103	192	194	60	54	202	139
4:00		76	49	97	104	19	54	198
5:00		114	103	97	89	98	112	161
6:00		165	208	106	221	53	57	30
7:00		155	311	191	143	72	51	10
8:00		358	210	105	235	135	314	46
9:00		195	122	210	138	225	149	
10:00		125	210	125	132	223	148	
11:00		187	121	150	77	251	94	
Noon	90	89	33	92	139	172	55	
1:00	54	46	58	31	150	98	54	
2:00	35	34	43	53	50	61	41	
3:00	85	77	19	55	29	22	41	
4:00	68	25	52	10	50	52	75	
5:00	66	00	34	76	21	41	78	
6:00	86	42	27	74	30	14	54	
7:00	31	24	38	54	86	34	38	
8:00	30	53	32	09	142	58	64	
9:00	65	24	00	21	76	42	86	
10:00	00	40	74	00	32	55	24	
11:00	58	23	121	21	97	245	202	

*The hourly percent of oxygen was computed by dividing the running average for each hour into the average hourly oxygen consumption.

TABLE VI

AN HOURLY % OF OXYGEN COMPUTED FROM RUN 2*

Time	Day 1 %	Day 2 %	Day 3 %	Day 4 %	Day 5 %	Day 6 %	Day 7 %	Day 8 %
Mdnt.		56	49	116	105	120	83	89
1:00		36	46	108	97	110	108	104
2:00		28	17	34	50	49	87	96
3:00		32	51	68	78	124	61	98
4:00		63	73	129	82	88	102	95
5:00		68	25	62	100	88	101	141
6:00		103	95	109	103	134	126	113
7:00		219	195	123	118	109	115	122
8:00		267	284	151	122	105	129	130
9:00		146	191	132	149	129	132	108
10:00		23	82	97	137	105	104	
11:00		73	100	93	108	84	86	
Noon	133	91	85	125	91	96	82	
1:00	123	202	116	90	93	94	100	
2:00	88	143	88	70	75	105	98	
3:00	124	125	91	108	80	68	91	
4:00	114	88	71	121	81	85	93	
5:00	93	88	97	80	104	106	109	
6:00	142	249	85	71	80	112	91	
7:00	112	98	92	114	82	76	83	
8:00	71	70	87	114	115	134	102	
9:00	76	52	120	95	98	104	105	
10:00	76	55	112	113	75	90	117	
11:00	76	62	101	96	129	131	105	

*The hourly percent of oxygen was computed by dividing the running average for each hour into the average hourly oxygen consumption.

TABLE VII

AN HOURLY % OF OXYGEN COMPUTED FROM RUN 3*

Time	Day 1 %	Day 2 %	Day 3 %	Day 4 %	Day 5 %	Day 6 %	Day 7 %	Day 8 %
Mdnt.		52	239	161	128	115	103	116
1:00		67	71	177	109	105	103	117
2:00		35	65	76	106	116	108	97
3:00		74	127	79	66	113	94	98
4:00		63	51	63	101	102	89	111
5:00		72	69	124	101	85	94	114
6:00		216	141	96	137	112	97	111
7:00		156	193	128	122	102	92	110
8:00		138	194	114	119	120	103	96
9:00		111	186	146	104	107	118	113
10:00		268	97	103	100	96	100	90
11:00		176	33	116	101	87	93	98
Noon	204	131	94	89	101	94	94	
1:00	54	46	35	78	99	96	88	
2:00	00	69	33	51	74	84	104	
3:00	00	23	32	77	73	96	113	
4:00	38	33	171	89	99	93	66	
5:00	71	58	89	108	101	94	87	
6:00	54	32	46	85	83	109	98	
7:00	38	71	96	72	97	97	77	
8:00	39	67	69	84	88	112	120	
9:00	00	78	120	88	99	116	82	
10:00	82	69	88	105	105	109	116	
11:00	121	165	114	115	107	102	136	

*The hourly percent of oxygen was computed by dividing the running average for each hour into the average hourly oxygen consumption.

TABLE VIII

AN HOURLY % OF OXYGEN COMPUTED FROM RUN 4*

Time	Day 1 %	Day 2 %	Day 3 %	Day 4 %	Day 5 %	Day 6 %	Day 7 %	Day 8 %
Mdnt.		140	116	101	149	115	102	92
1:00		115	108	113	78	93	72	95
2:00		106	124	119	107	109	97	97
3:00		83	123	101	92	97	104	108
4:00		100	111	114	89	95	109	112
5:00		106	105	95	95	87	95	84
6:00		125	115	113	121	103	130	121
7:00		98	119	114	123	113	104	124
8:00		133	116	120	113	126	112	
9:00		146	116	113	111	161	126	
10:00		126	122	95	108	107	114	
11:00		86	106	109	88	98	101	
Noon	100	97	103	103	81	119	130	
1:00	78	78	75	93	95	82	95	
2:00	66	90	73	79	102	115	93	
3:00	107	80	78	90	90	98	92	
4:00	88	56	73	97	77	98	92	
5:00	71	63	88	79	81	90	72	
6:00	66	77	95	72	67	76	102	
7:00	59	73	75	93	91	87	67	
8:00	68	86	99	95	124	130	96	
9:00	57	80	69	87	75	63	92	
10:00	125	126	113	113	113	115	51	
11:00	125	137	121	117	122	123	82	

*The hourly percent of oxygen was computed by dividing the running average for each hour into the average hourly oxygen consumption.

$$\frac{\frac{\sum X_1 \quad X_{24}}{24} + \frac{\sum X_2 \quad X_{25}}{24}}{2} = A_1$$

$$\frac{X_{13}}{A_1} = H_1\%$$

The formulas given above are the ones used to compute the running averages (A_n) and hourly percents (H_n). X_n stands for the hourly differences of oxygen consumption as given in Tables I, II, III, and IV. The hourly differences of oxygen consumption (X) are averaged for a 24 hour period giving one running average (A). This average is divided into the middle number of the 24 numbers used to obtain the average in order to get the relative percent of oxygen consumption above or below the average consumption for that 24 hour period. Since the number 24 has no middle number, it was necessary to average one 24 hour period and then average a second 24 hour period by beginning an hour later than the beginning of the first 24 hour average. By combining these two 24 hour averages and dividing by 2, an average of the two may be obtained. The thirteenth hour of the first 24 hour average or the twelfth hour of the second 24 hour average (they are the same number) is the middle number. By shifting one hour and making new 24 hour averages so that the middle number is one hour later than the previous

middle number, the percent of oxygen consumption above or below the average may be obtained for that hour. This procedure was repeated for all the hours of data and is given in Tables V, VI, VII and VIII. These relative percents of oxygen consumption make up the data that is used to compare and make all correlations in this paper. Because of the mechanics of the mathematics, no percents for the first 12 hours or the last 12 hours of the eight day run were determined.

A review of what was said previously would clarify the analysis. A biological clock should persist in constant conditions at a period of approximately 24 hours but not necessarily an exact 24 hours. In fact an exact 24 hour cycle may indicate that there was still some synchronizer in effect, and the particular periodicity observed was still being regulated by this external synchronizer, therefore, no biological clock.

The method used to determine if there was a periodicity present or not, was to examine the first three days of each run for similarities when the LD 12:12 was in effect. The following five days of DD were examined to determine if the similarities were persistent. A persistent high or low oxygen consumption for a particular hour of the day was one similarity that occured. No satisfactory method

could be found to statistically analyze the data for persistent peaks or dips at certain hours of the day. Correlation of the daily polymodal graphs had to be by inspection of graphs. One typical 8 day run is shown in Figures 4 and 5. These graphs were made from Run 2 (Table V). Figure 4 has the three days of LD and Figure 5 has the five days of DD. Each of the days were given a different symbol so that they could be distinguished. Graphs of this nature were made for all the runs and analysis of data was made from them.

The three runs with the light cycle beginning at 8:35 pm and ending at 8:50 am showed a large increase in oxygen consumption from 6:00 am to 10:00 am. This large increase in oxygen consumption was evident for the three days of LD and in the succeeding five days of DD. There was a lesser amount of increase in oxygen consumption during the DD period.

This was the major or most overt periodicity in all of the eight day runs. Why this high increase came near the end of the light period was not clear. An observation of the dense mycelia rings appeared at the end of the light cycle, but the formation of these dense rings was so gradual that no strict conclusions could be drawn as to exactly when they were formed. It cannot be concluded that the formation of these mycelia rings were the cause of the extremely large

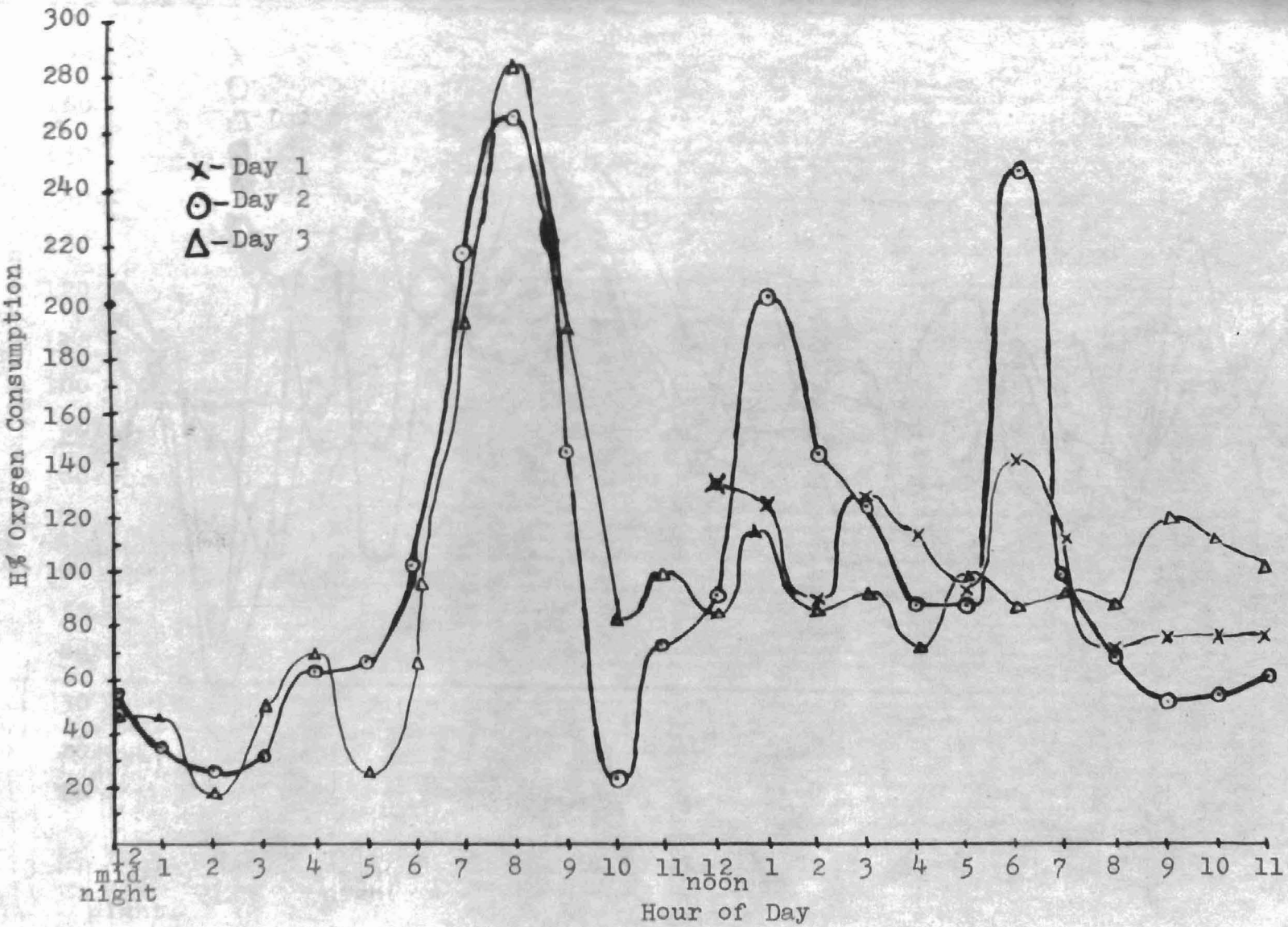

Figure 4. Oxygen consumption for the first three days of run 2 (LD 12:12).

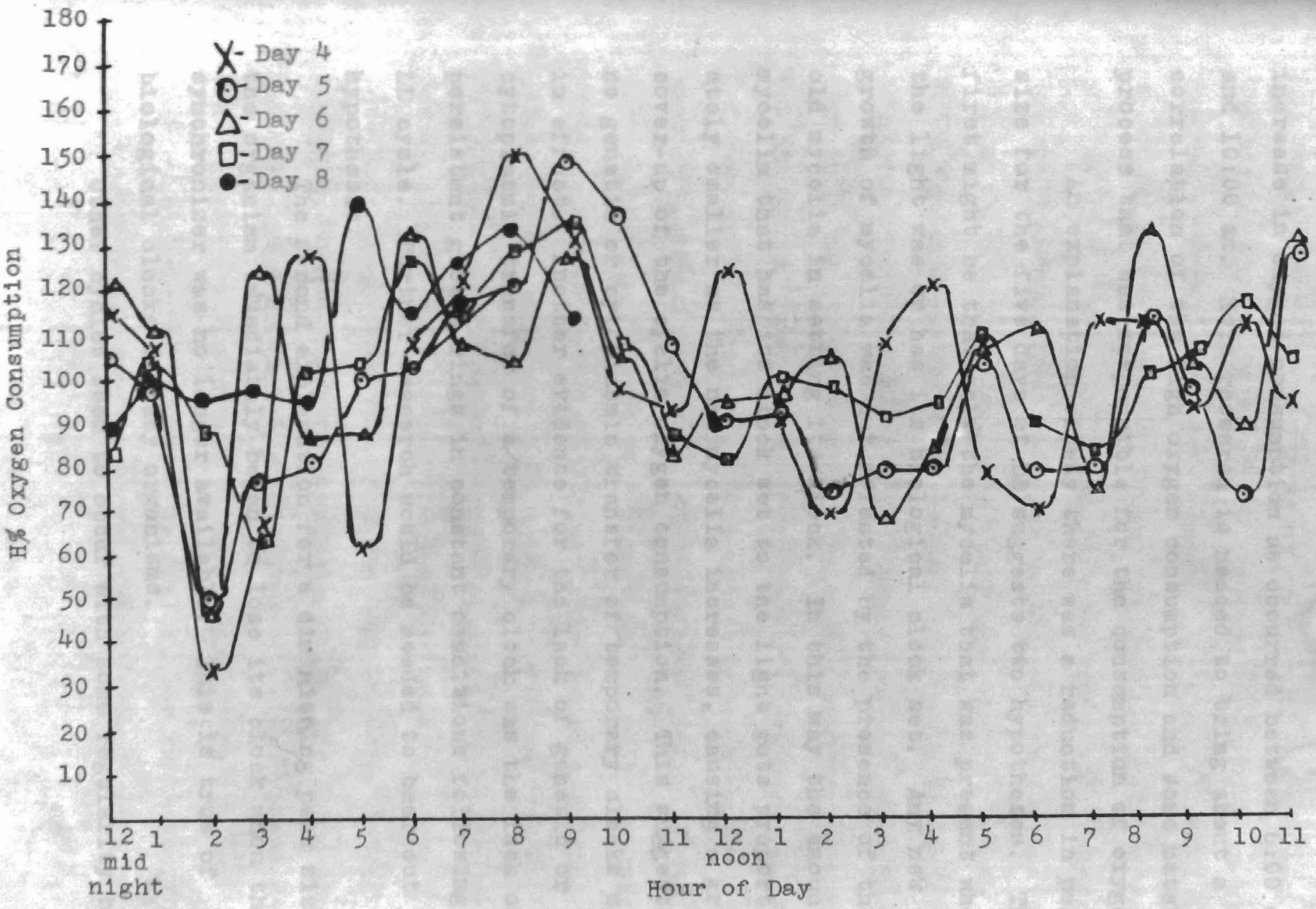

Figure 5. Oxygen consumption for the last five days of run 2 (DD).

increase in oxygen consumption as occurred between 6:00 am and 10:00 am. More research is needed to bring about a correlation of the high oxygen consumption and some metabolic process that was responsible for the consumption of oxygen.

An explanation of why there was a reduction in peak size for the five days of DD suggests two hypotheses. The first might be that only the mycelia that was present when the light was on has its biological clock set. Any new growth of mycelia was not affected by the presence of the old mycelia in setting its clock. In this way the amount of mycelia that has its clock set to the light gets proportionately smaller as the new mycelia increases, causing a gradual cover-up of the cyclic oxygen consumption. This suggests that no genetic or cytoplasmic transfer of temporary clocks was in effect. Another evidence for the lack of genetic or cytoplasmic transfer of a temporary clock was the lack of persistent growth rings in constant conditions following a LD cycle. Further research would be needed to bear out this hypothesis.

The second explanation for a diminishing peak size was the organism immediately began to lose its clock when the synchronizer was no longer available. This is true of biological clocks in many organisms.

Other cycles seem to occur with some regularity but

not as consistently as the major peak mentioned before. One was a very sharp dip in oxygen consumption at 7:00 am or 8:00 am and more rarely at 9:00 am. This dip could be found only during the 5 days of DD and the 2nd day of LD but never on the third day of LD. The first day could not be determined because the mechanics in computing the running average caused a loss of the first twelve hours of day one.

Another observation was made concerning the interval of peaks of oxygen consumption. The number of hours between each peak was recorded and an arithmetic mean was obtained for the first three runs. There was an average of 3.2 hours between peaks. From the 149 intervals between peaks, there were 49 two hour intervals, 47 three hour intervals, 32 four hour intervals, 14 five hour intervals and only 6 intervals of six or more hours. This clearly indicated a predominance of two, three, and four hour intervals.

Because the peaks recurred at an average of three hours, it was natural that there should be many minor crests that were in close correlation from day to day, therefore, periodic. These minor recurring peaks, as the large crests had a tendency to do, shifted one hour one way or the other from one day to the next. It was difficult to say when a peak should occur. Speaking generally, the peaks occurred every two, three, or four hours of the day and could be

found at the same hour as the day before or with no more than a one hour shift.

A large crest occurred at 11:00 pm or 12:00 pm during the light cycle. Because two large crests, 11:00 pm to 12:00 pm and 6:00 am to 10:00 am occurred during the light cycle, the average oxygen consumption was larger during the light phase than the dark phase. Calculations were made by taking the arithmetic mean of percents of oxygen consumption during the light hours and then the dark hours for all days of all runs. During the light hours average consumption per hour was 113% and during the dark hours the average consumption was 86%.

Analysis of the data from run 4 using light cycles reversed from runs 1, 2, and 3 indicated that light was not the primary synchronizer. It was assumed that if light was the primary synchronizer, the data of run 4 should have shifted 180% to correlate with the shifted light. Essentially no shift occurred. The major crest occurring between 6:00 am and 10:00 am continued to occur during the same time in run 4. The only evidence of a shift was a large crest at 8:00 pm and at 11:00 pm or 12:00 pm. Both of these crests are present in the first three runs, but comparatively not as large as run 4.

The average oxygen consumption for the light and dark periods were computed from the percents of oxygen

consumed as they were for the first three runs. From 9:00 am to 8:00 pm the average oxygen consumption was 92.5% and from 9:00 pm to 8:00 am it was 106.0%. The high and low oxygen consumption for the same 12 hour periods of the first three runs were relatively the same.

The average interval of time between peaks was 3.4 hours as compared to 3.2 hours in the first three runs. The occurences of 2-3-4 hour intervals were essentially the same with 14 two hour intervals, 13 three hour intervals, 10 four hour intervals and 11 intervals of five or more hours.

The data from the fourth run indicated that light was not the primary synchronizer. It had a small influence on the rhythmic pattern by increasing the oxygen consumption at 9:00 pm and at 11:00 pm to 12:00 pm. The fact that light had an effect on oxygen consumption was noticed in all runs. Very large increases in oxygen consumption were found in the first three days of LD. The last five days of DD always had relatively smaller increases in oxygen consumption. But the major pattern of oxygen consumption in run 4 remained unchanged from the first three runs. This was indicated by the major peak of oxygen consumption in run 4 occurring from 6:00 am to 10:00 am as it did in the first three runs.

Another indication that run 4 remained essentially unchanged was the high average of oxygen consumption that

occurred from 9:00 pm to 9:00 am as opposed to the low average that occurred from 9:00 am to 9:00 pm. This indicated that another synchronizer or possible several synchronizers may have been more directly responsible for the primary oxygen consumption. The other synchronizer or synchronizers may be any of the geophysical phenomena as suggested by F. A. Brown, Jr. Because the other synchronizer or synchronizers are unknown, it is impossible to determine if the "constant" conditions in which the experiment was run for the five days DD eliminated the synchronizer. This makes impossible the determination whether Ceratocystis ulmi had an internal biological clock or an external, continuously synchronized, periodicity.

In conclusion, Ceratocystis ulmi was grown in constant temperatures for eight days, the first three days in a LD 12:12 and then a continuous darkness for five days. An analysis of all the data showed a definite periodicity in the organism. This periodicity was partially synchronized by light. This was determined when the light pattern was reversed. The primary periodic oxygen consumption for the normal light pattern and the reversed light pattern remained the same with two small exceptions. For example; the oxygen consumption increased at 9:00 pm and 11:00 pm to 12:00 pm and it was indicated the light played a minor role in

synchronizing the organism. Since the main synchronizer was unknown, it could not be determined if the main periodicity was internal, a biological clock, or external, a continuously synchronized periodicity. More research is required to determine the main synchronizer.

CHAPTER V

SUMMARY

A search of the literature indicated a great variety of organisms used in research of biological clocks. Little has been done with fungi and no study had been done using Ceratocystis ulmi.

Measurements were made by growing Ceratocystis ulmi in a diver similar to a Cartesian diver. The diver was placed in water and gradually sunk as oxygen was consumed by the organism. The sinking was recorded by a series of levers attached at one end to the diver. The other end of the levers scribed a line on a drum that revolved once every 24 hours. The pen movements were considered a record of oxygen consumption. Measurements were made to determine the hourly movements of the pens. The organism was grown for eight days in the diver. For the first three days an alternating 12 hour light, 12 hour dark cycle was used. For the last five days, the organism was grown in complete darkness. Mathematical computations were made to adjust the data for the growth factor of the organism.

Periodicity of oxygen consumption was evident. A high oxygen consumption occurred every 24 hours between 6:00 am and 10:00 am. Another evidence for periodicity in the organism was high oxygen consumption occurring

approximately every three hours. More oxygen was consumed during the hours of 9:00 pm to 9:00 am than at 9:00 am to 9:00 pm. A periodic oxygen consumption was partially synchronized by light in each experiment. This was evidenced by high increases in oxygen consumption during the first three days of LD and low increases in oxygen consumption during the last five days of DD. A one hundred and eighty degree shift in the normal light pattern brought about a small shift in the oxygen consumption. The main cycles of oxygen consumption remained unchanged. This indicates that light was a minor synchronizer. Some other synchronizer or synchronizers were synchronizing the main periodicity. Because the main synchronizer was unknown, no conclusion could be drawn to determine if the major mechanism for periodicity was biologically timed or externally timed.

Continued research in determining the identification of the synchronizer is recommended.

BIBLIOGRAPHY

BIBLIOGRAPHY

A. PUBLICATIONS OF THE GOVERNMENT, LEARNED SOCIETIES, AND OTHER ORGANIZATIONS

Pittendrigh, C. S. and V. G. Bruce. "Daily Rhythms as Coupled Oscillator Systems and their Relation to Thermoperiodism and Phototropism," Photoperiodism and Related Phenomena in Plants and Animals, pp. 475-505, C. Withrow, editor, Washington: American Association for the Advancement of Science, 1959.

B. PERIODICALS

Barnwell, Franklin H. "Observations and Daily Tidal Rhythms in Some Fiddler Crabs from Equatorial Brazil," Biological Bulletin, CXXV (December, 1963), 399-415.

Brown, F. A. Jr. "A Simple Automatic, Continuous-Recording Respirometer," Review of Scientific Instruments, (1954b), 415-417.

Brown, Frank A. Jr. "An Exogenous Reference Clock for Persistent Temperature-Independent, Liable Biological Rhythms," Biological Bulletin, CXV (August, 1958), 81-100.

Brown, F. A. Jr. "Extrinsic Rhythmicality: A Reference Frame for Biological Rhythms under so Called Constant Conditions," Annals of the New York Academy of Sciences, XCVIII (October, 1962), 775.

Brown, F. A. Jr. "Responses of the Planarian, Dugesia and the Protozoan Paramecium, to Very Weak Horizontal Magnetic Fields," Biological Bulletin, CXXIII (October, 1962), 264-281.

Brown, Frank A. Jr. "Responses of the Planarian, Dugesia to Very Weak Horizontal Electrostative Fields," Biological Bulletin, CXXIII (October, 1962), 282-294.

Brown, Frank A. Jr., J. Shriner and C. L. Ralph. "Solar and Lunar Rhythmocity in the Rat in Constant Conditions and the Mechanism of Physiological Time Measurement," American Journal of Physiology, CLXXXLV (March, 1956), 491-496.

Bruce, V. G. and C. S. Pittendrigh. "Resetting the Euglena Clock with a Single Light Stimulus," American Naturalist, XCII (September-October, 1958), 295-306.

Hastings, J. W. "Unicellular Clocks," Annual Review of Microbiology, XIII (November, 1959), 397-312.

Hastings, J. W. and B. M. Sweeney. "A Persistent Diurnal Rhythm of Luminescence in Gonyaulax poledra," Biological Bulletin, CXV (December, 1958), 440-448.

Hunt, John. "Taxonomy of Genus Ceratocystis," Lloydia, XIX (January, 1958), 1-58.

Maller and Dieter. "Development of Sporangia and Lunar Periodicity on Brown Algae," Botanical Marina, IV (February, 1962), 140-162.

Moreau, C. "Ceratocystis ulmi," Revuede Mycologie Supplementary Colonial, XVII (January, 1952), 22.

Pittendrigh, Colin S., V. G. Bruce, N. S. Rosenwerg and M. L. Rubin, "Growth Patterns in Neurospora," Nature, CLXXXLV (July 18, 1959), 169-171.

Ralph, C. L. "Modifications of Activity Rhythms of Periplaneta americana (L) Induced by Carbon-Dioxide and Nitrogen," Physiological Zoology, XXXII (January, 1959), 57-62.

Schmidle, A. "Die Tagesperiodizitat der Asexuellen Reproduktion von Pilobolus sphaerosporus," Archiv fur Mikrobiologie, XVI (May, 1959), 80-100.

Uebelmesser, E. R. "Ueberden endonomer Tagesrhythmus der Sporangientraegerbildung von Pilobolus," Archiv fur Mikrobiologie, XX (February, 1954), 1-33.

C. ESSAYS AND ARTICLES IN COLLECTIONS

Aschoff, Jurgen. "Exogenous and Endogenous Components in Circadian Rhythms," Biological Clocks, Leonora Frisch, editor. Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960. Pp. 11-129.

Brown, F. A. Jr. "Response to Pervasive Geophysical Factors and the Biological Clock Problem," Biological Clocks, Leonora Frisch, editor. Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960. Pp. 297-312.

Bruce, Victor G. "Environmental Entrainment of Circadian Rhythms," Biological Clocks, Leonora Frisch, editor. Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960. Pp. 29-45.

Bunning, E. "Endogenous Diurnalcycles of Activity in Plants," Rhythmic and Synthetic Processes in Growth, G. Rudnik, editor. Princeton: Princeton University Press, 1957. Pp. 111-126.

Bunning, E. "Opening address: Biological Clocks," Biological clocks, Leonora Frisch, editor. Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960. Pp. 1-9.

Lobban, M. C. "The Entrainment of Circadian Rhythms in Man," Biological Clocks, Leonora Frisch, editor. Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960. Pp. 325-332.

Pittendrigh, Collin S. "Circadian Rhythms and the Circadian Organization of Living Systems," Biological Clocks, Leonora Frisch, editor. Symposia on Quantitative Biology, Volume XXV, Long Island, New York: Long Island Biological Association, 1960. Pp. 159-184.

Wilkins, Malcolm B. "The Effect of Light Upon Plant Rhythms," Biological Clocks, Leonora Frisch, editor. Symposia on Quantitative Biology, Volume XXV. Long Island, New York: Long Island Biological Association, 1960. Pp. 115-129.